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Crystallization and preliminary X-ray diffraction analysis of creatine amidinohydrolase from *Actinobacillus*

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The homodimeric form of creatine amidinohydrolase from *Actinobacillus* has been crystallized by the hanging-drop vapour-diffusion method followed by macroseeding using PEG 6000 as a precipitant. The crystals belong to space group *I*222, with unit-cell parameters a = 111.26 (3), b = 113.62 (4), c = 191.65 (2) Å, and contain two molecules in an asymmetric unit. A complete diffraction data set using synchrotron radiation was collected to 2.7 Å resolution.

1. Introduction

Creatine is found in the blood, brain and muscle of many living organisms. Creatinine occurs in muscle cells as the only intermediate product of creatine metabolism. In the metabolic degradation pathway of creatinine, creatininase (EC 3.5.2.10; creatinine amidohydrolase) is the first enzyme in the pathway that hydrolyses creatinine to creatine, which is subsequently transformed into sarcosine and urea in a second hydrolyzation step by creatinase (EC 3.5.3.3; creatine amidinohydrolase). Sarcosine is demethylated by sarcosine oxidase (EC 1.5.3.1) or sarcosine dehydrogenase (EC 1.5.99.1) to produce glycine and formaldehyde (Tsuru *et al.*, 1976). Measurement of the creatinine levels in serum and determination of the renal clearance of creatinine are widely used for laboratory diagnosis of renal and muscular function (Madaras & Buck, 1996). Both compounds, creatinine and creatine, can be used by microorganisms as carbon and nitrogen sources (Tsuru, 1977). The enzyme creatinase was first characterized by Roche *et al.* (1950), who showed that in two *Pseudomonas* species (*P. eisenbergii* and *P. ovalis*) creatine is metabolized to urea. Creatinase is also found in other bacteria, such as *P. putida* DSM2106 (Hoeffken *et al.*, 1988), *Flavobacterium* sp. U-188 (Koyama *et al.*, 1990) and *Bacillus* sp. B-0618 (Suzuki *et al.*, 1993) and plays a general role in microbial creatinine degradation.

The crystal structure analysis and enzymatic mechanism of creatinase from *P. putida* have been reported (Hoeffken *et al.*, 1988; Coll *et al.*, 1990). To our knowledge, this is the only creatinase protein structure available in the PDB. *Actinobacillus* are Gram-negative rod-shaped aerobic and parasitic bacteria. They are pathogenic for animals and are hence clinically important because of their resistance to most antibiotics. Since creatinine and creatine can be used by microorganisms as carbon and nitrogen sources, creatinase may play a role in the disease process caused by *Actinobacillus*. We have therefore initiated crystallographic studies of creatinase from *Actinobacillus* to understand its functional role in *Actinobacillus* and also to investigate the structural relationships of creatinases at the tertiary structural level. We report here the crystallization and preliminary crystallographic study of *Actinobacillus* creatinase.

2. Materials and methods

2.1. Crystallization

Actinobacillus creatinase protein was purchased from Toyoboseki Ltd (Japan). The protein was dialysed against a buffer of 20 m*M* sodium citrate pH 5.5 and concentrated to approximately 10.0 mg ml^{-1}. Before starting crystallization trials, mass-spectrum analysis using the method of matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy (MALDI-TOF MS; PerSeptive Inc.) was carried out to check the purity of the protein. It gave a single peak corresponding to a monomer molecular weight of 46.5 kDa. Initial crystallization trials using the hanging-drop vapour-diffusion method and a wide range of conditions (including Hampton Research Screens I and II) were tried. Drops containing equal volumes (2 µl each) of protein and reservoir solution were equilibrated against 0.5 ml of reservoir solution. Microcrystalline precipitates were generally found in the presence of PEG 4000 as a major precipitant. Further fine screening was carried out using different PEGs (PEG 1000, PEG 4000, PEG 6000, PEG 8000, PEG MME 5000) over a wide pH range. Tiny crystals grew in a drop containing 15–20% PEG 6000, 0.1 *M*

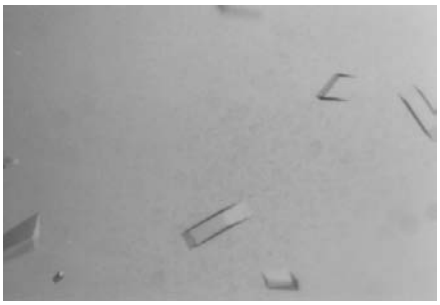

Figure 1
Crystals of creatine amidinohydrolase from *Actinobacillus*.

Table 1
Data-collection and processing statistics.

Values in square brackets are for the highest resolution shell (2.85–2.70 Å).

Space group	*I*222
Unit-cell parameters (Å)	*a* = 111.26 (3), *b* = 113.62 (4), *c* = 191.65 (2)
Resolution range (Å)	30.0–2.7
No. of measured reflections	167670
No. of unique reflections	33902
$R_{\rm merge}$† (%)	6.5 [15.1]
Completeness (%)	99.8 [99.9]
Average $I/\sigma(I)$	10.6 [4.9]

† $R_{\rm merge} = \sum|I(h) - \langle I(h)\rangle|/\sum I(h)$, where $I(h)$ is the observed intensity and $\langle I(h)\rangle$ is the mean intensity of reflection h over all measurements of $I(h)$.

sodium citrate pH 5.0. These crystals were crushed and used for microseeding. Crystals appearing in seeded drops are mainly twinned and also do not grow to a size sufficient for X-ray diffraction. Hence, carefully selected untwinned crystals from these drops were used for macroseeding. Crystals of approximate dimensions 0.25 × 0.2 × 0.06 mm (Fig. 1) were obtained in a solution containing 10% PEG 6000, 0.1 *M* sodium citrate pH 5.0.

2.2. Data collection

The diffraction of the crystals was initially tested on an R-AXIS IV system mounted on a Rigaku rotating-anode generator equipped with a double-mirror focusing system, operated at 40 kV and 90 mA. The crystals diffracted beyond 3.0 Å resolution, but we were unable to collect a complete data set on the R-AXIS IV because of sustained radiation damage. Hence, data collection was carried out from a single crystal on beamline BL18B using the ADSC Quantum-4 CCD detector at the Photon Factory, Tsukuba, Japan. The wavelength used was 1.00 Å and the incident beam was collimated to 0.1 mm in diameter. The crystal-to-detector distance was set to 200 mm. A complete data set was collected at room temperature to a maximum resolution of 2.7 Å (Fig. 2). All data were processed and scaled using the programs *DPS*/*MOSFLM* (Rossmann & van Beek, 1999) and *SCALA* from the *CCP*4 package (Collaborative Computational Project, Number 4, 1994).

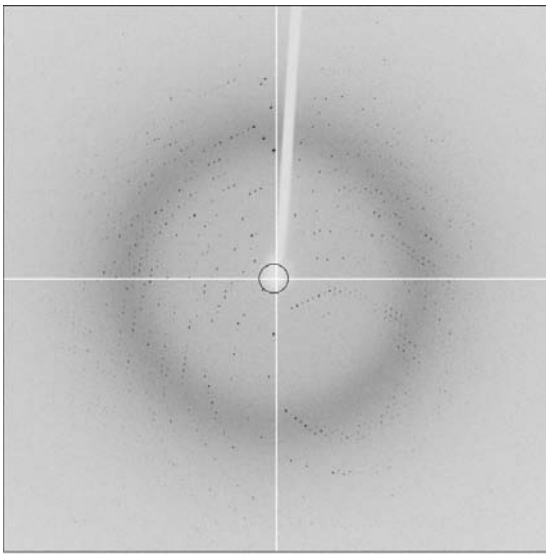

Figure 2
An X-ray diffraction pattern from an *Actinobacillus* creatinase crystal (oscillation range 1.0°).

3. Results

Crystals with dimensions of 0.25 × 0.2 × 0.06 mm were obtained by macroseeding. Examination of the diffraction data from the creatinase crystals revealed that these crystals diffract to 2.7 Å resolution and belong to the orthorhombic space group *I*222, with unit-cell parameters *a* = 111.26 (3), *b* = 113.62 (4), *c* = 191.65 (2) Å. Data-collection statistics are summarized in Table 1. A total of 167 670 measured reflections were merged into 33 902 unique reflections with an $R_{\rm merge}$ of 6.5%. The merged data set was 99.8% complete to 2.7 Å resolution.

The amino-acid sequence of *Actinobacillus* creatinase is not yet known. Mass-spectral studies suggest that the molecular weight of this protein is 46.5 kDa. This molecular size fits well with that of other known creatinases. Based on this value, a Matthews coefficient of 3.26 $Å^3\,Da^{-1}$ (Matthews, 1968) suggests a dimer in the asymmetric unit and a solvent content of 62%. The *Actinobacillus* creatine amidinohydrolase protein sequence determination and structure determination by the molecular-replacement method are now in progress.

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